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[0008] The method of the present invention is directed at stimulating bone growth in a subject and can be used at sites where bone growth would not occur, absent treatment with autologous bone grafts or administration of bone growth factors. The method involves the administration of agonists of the non-proteolytic thrombin receptor. Such agonists include small peptides and physiologically functional equivalents having homology to the segment between amino acid 508 and 530 of human prothrombin. These small peptides are inexpensive to prepare in bulk quantities and are osteoinductive at low dose. In addition, their lyophilized form is stable for at least thirty months when stored at 5.degree. C. and at 60% relative humidity.

DETAILED DESCRIPTION OF THE INVENTION

[0009] "Osteoinduction" refers to stimulating bone growth at a site within a subject at which little or no bone growth would occur if the site were left untreated. Sites which could therapeutically benefit from the induction of bone growth are referred to as "in need of osteoinduction". Examples include non-union fractures or other severe or massive bone trauma. It is noted that bone growth normally occurs at bone injuries such as simple or hairline fractures and well opposed complex fractures with minimal gaps without the need for further treatment. Such injuries are not considered to be "in need of osteoinduction".

[0010] Simple fracture repair appears to be quite different from the induction of bone formation required to fill non-union fractures, segmental gaps or bone voids caused, for example, by removal of a bone tumor or cyst. These cases require bone grafting or induction of new bone growth generally employing some type of matrix or scaffolding to serve as a bone growth substitute. Induced bone growth can also be therapeutically beneficial at certain sites within a subject (referred to as "ectopic" sites) where bone tissue would not normally be found, such as a site in need of a bone graft or bone fusion. Fusions are commonly used to treat lower back pain by physically coupling one or more vertebrae to its neighbor. The bone created by such a fusion is located at a site not normally occupied by bone tissue. Osteoinduction at these ectopic sites can act as a "graft substitute" whereby induced bone growth between the vertebrae takes the place of a graft and obviates the need for a second operation to harvest bone for the grafting procedure. Induction of bone growth is also needed for treating acquired and congenital craniofacial and other skeletal or dental anomalies (see e.g., Glowacki et al., Lancet 1: 959 (1981)); performing dental and periodontal reconstructions where lost bone replacement or bone augmentation is required such as in a jaw bone; and supplementing alveolar bone loss resulting from periodontal disease to delay or prevent tooth loss (see e.g., Sigurdsson et al., J. Periodontol., 66: 511(1995)).

[0011] Applicants have discovered that compounds which stimulate or activate the non-proteolytically activated thrombin receptor (hereinafter "NPAR") are osteoinductive. Such compounds are said to be NPAR agonists. NPAR is a high-affinity thrombin receptor present on the surface of most cells. This NPAR component is largely responsible for high-affinity binding of thrombin, proteolytically inactivated thrombin, and thrombin derived peptides to cells. NPAR appears to mediate a number of cellular signals that are initiated by thrombin independent of its proteolytic activity. An example of one such signal is the upregulation of annexin V and other molecules identified by subtractive hybridization (see Sower, et. al., Experimental Cell Research 247:422 (1999)). NPAR is therefore characterized by its high affinity interaction with thrombin at cell surfaces and its activation by proteolytically inactive derivatives of thrombin and thrombin derived peptide agonists as described below. NPAR activation can be assayed based on the ability of molecules to stimulate cell proliferation when added to fibroblasts in the presence of submitogenic concentrations of thrombin or molecules that activate protein kinase C or compete with .sup.125I-thrombin for high affinity binding to thrombin receptors, as disclosed in U.S. Pat. Nos. 5,352,664 and 5,500,412 and in Glenn et al., J. Peptide Research 1:65 (1988). NPAR is to be distinguished from other thrombin binding proteins and the cloned family of proteolytically-activated receptors for thrombin, including the receptors PAR1, PAR2, PAR3 and PAR4. PAR1 possesses a specific thrombin cleavage site that allows thrombin cleavage to expose a new amino-terminus domain that acts as a tethered ligand folding back onto itself inducing its activation (see, Vu, et al., Cell. 64:1057 (1991)). PAR2 has a similar